BIO-RAD

# ddPCR Supermix for Probes

| Catalog # | Description |
|---|---|
| 1863026 | **ddPCR Supermix for Probes**, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions |
| 1863010 | **ddPCR Supermix for Probes**, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions |
| 1863027 | **ddPCR Supermix for Probes**, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions |
| 1863028 | **ddPCR Supermix for Probes**, 50 ml (10 x 5 ml vials), 5,000 x 20 µl reactions |

**For research purposes only.**

## Description

ddPCR Supermix for Probes is a 2x concentrated, ready-to-use reaction cocktail containing all components — except primers, probe(s), and template — required for probe-based Droplet Digital PCR (ddPCR). The mixture delivers maximum target specificity and fluorescence amplitude and minimum droplet variability to ensure precise target quantification.

The hot-start feature of the polymerase in the supermix enables partitioning of sample into droplets while keeping the enzyme inactive at ambient conditions. The supermix has been optimized to support the amplification and detection of DNA targets using commercially available probe-based assays and is suitable for use with uracil N-glycosylase (UNG) for PCR decontamination. UNG may be purchased from a licensed supplier.

## Storage and Stability

ddPCR Supermix for Probes is stable at –20°C through the expiration date printed on the label. Once thawed, it can be stored at 4°C for up to 2 weeks. Repeated freezing and thawing of the supermix is not recommended.

## Quality Control

ddPCR Supermix for Probes is free of contaminating DNase and RNase. Stringent specifications are maintained to ensure lot-to-lot consistency.

## Recommendations for Optimal Results

- Follow general guidelines and recommendations for Droplet Digital PCR (refer to the Droplet Digital PCR Applications Guide, bulletin 6407)
- The concentration of intact human genomic DNA should be ≤66 ng per 20 µl reaction. If using higher concentrations, digest DNA with a restriction endonuclease (see guidelines in DNA Digestion section)

## Required Equipment

The QX200 Droplet Digital PCR System (catalog #1864001), QX200 AutoDG Droplet Digital PCR System (#1864100), or QX ONE Droplet Digital PCR System (#12006536) is required.

Refer to the QX200 Droplet Reader and QuantaSoft Software and QX200 Droplet Generator Instruction Manuals (10031906 and 10031907, respectively), the Automated Droplet Generator Instruction Manual (10043138), or the QX ONE Droplet Digital PCR System Instruction Manual (10000116512) for ordering information about consumables, such as oils, cartridges, gaskets, plates, and seals.

## Reaction Setup

1. Thaw all components to room temperature. Mix thoroughly by vortexing each tube to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of the tubes.
2. Prepare samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix for the number of reactions needed according to the guidelines in Table 1. Assemble all required components except the sample, dispense equal aliquots into each reaction tube, and add the sample to each reaction tube as the final step.

**Table 1. Preparation of the reaction mix.**

| Component | Volume per Reaction, µl | Final Concentration |
|---|---|---|
| 2x ddPCR Supermix for Probes | 10 | 1x |
| 20x target primers/probe (FAM or Cy5)* | 1 | 900 nM/250 nM |
| 20x target primers/probe (HEX/VIC or Cy5.5)* | 1 | 900 nM/250 nM |
| Diluted restriction enzyme (see DNA Digestion section) | 1 | Variable |
| Sample | Variable | Up to 330 ng** |
| RNase-/DNase-free water | Variable | – |
| **Total volume*** ** | **20** | **–** |

* Cy5 and Cy5.5 channels are available only on the QX ONE ddPCR System.
** Sample concentrations >66 ng per reaction and certain applications may require restriction digestion for optimal target detection. If digestion is not required, prepare the ddPCR reaction mix without the diluted restriction enzyme.
*** For the Automated Droplet Generator, prepare 22 µl per reaction.

4. Mix thoroughly by vortexing the tubes. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes. Allow reaction tubes to equilibrate at room temperature for about 3 min.

5. Transfer the reaction mix from the reaction tubes to the appropriate ddPCR Cartridge as follows:
   - For the QX200 Droplet Digital PCR System, load 20 µl of each reaction mix into a sample well of a DG8 Cartridge. Follow subsequent instructions as specified in the QX200 Droplet Generator Instruction Manual (10031907)
   - For the QX200 AutoDG Droplet Digital PCR System, follow instructions in the Automated Droplet Generator Instruction Manual (10043138)
   - For the QX ONE Droplet Digital PCR System, load 20 µl of each reaction mix into a sample well of a GCR96 Cartridge. Follow subsequent instructions as specified in the QX ONE Droplet Digital PCR System Instruction Manual (10000116512)

### DNA Digestion (recommended)

DNA fragmentation by restriction digestion prior to droplet generation enables optimal accuracy by separating tandem gene copies, reducing sample viscosity, and improving template accessibility for input samples >66 ng per well. Choose a restriction endonuclease that does not cut either the target or reference amplicon and that is insensitive to methylation. Four-base cutters and high-fidelity enzymes are preferred.

Two strategies may be used to perform restriction digestion of DNA samples: digestion directly in the ddPCR reaction during setup, or conventional digestion prior to Droplet Digital PCR.

#### Digestion in ddPCR Reaction

- Dilute the restriction enzyme using the recommended diluent buffer according to the manufacturer's instructions, and add 1 µl to the ddPCR reaction as outlined in Table 1
- Approximately 2–5 units of restriction enzyme per 20 µl ddPCR reaction are recommended
- The addition of restriction enzyme buffers with high salt can inhibit Droplet Digital PCR and should be avoided
- HaeIII, MseI, AluI, HindIII, and CviQI have been observed to work well for digestions in ddPCR reactions

#### Digestion Prior to Droplet Digital PCR

- Use 5–10 enzyme units per microgram DNA, and 10–20 enzyme units per microgram genomic DNA
- Incubate the reaction for 1 hr at the temperature recommended for the restriction enzyme
- Heat inactivation is not required, but can be considered if long-term storage is required; do not heat inactivate above 65°C
- DNA purification is not necessary after restriction digestion
- Use a minimum 10-fold dilution of the digest to reduce the salt content of the sample in the ddPCR reaction
- Store digested DNA at –20°C or below
- Prepare the ddPCR reaction mix, as outlined in Table 1, without the diluted restriction enzyme

### Thermal Cycling Conditions

Follow instructions based on the system in use:

- For the QX200 Droplet Digital PCR System, carefully transfer droplets into a clean 96-well plate. Seal the plate using the PX1 PCR Plate Sealer at 180ºC for 5 sec. Proceed to thermal cycling (see Table 2)
- For the QX200 AutoDG Droplet Digital PCR System, seal the 96-well plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling (see Table 2)
- For the QX ONE Droplet Digital PCR System, use appropriate thermal cycling conditions as specified in Table 2 during plate setup. Refer to the QX ONE Droplet Digital PCR System Instruction Manual (10000116512) for plate setup instructions

**Table 2. Thermal cycling conditions.***

| Cycling Step | | Temperature, °C | Time | Number of Cycles |
|---|---|---|---|---|
| Hold (QX ONE ddPCR System only) | | 25 | 3 min | 1 |
| Enzyme activation | | 95 | 10 min | 1 |
| Denaturation | | 94 | 30 sec | 40 |
| Annealing/extension | | 60 | 1 min** | 40 |
| Enzyme deactivation | | 98 | 10 min | 1 |
| Hold | QX200 ddPCR System (optional) | 4 | Infinite | 1 |
| | QX ONE ddPCR System (required) | 25 | 1 min | 1 |

* For the C1000 Touch Thermal Cycler, use a heated lid set to 105°C and set the sample volume to 40 µl.

** Check/adjust ramp rate settings to ~2°C/sec.

### Data Acquisition and Analysis

Follow instructions based on the system in use:

- For the QX200 Droplet Digital PCR System and the QX200 AutoDG Droplet Digital PCR System, refer to the QX200 Droplet Reader and QuantaSoft Software Instruction Manual (10031906)
- For the QX ONE Droplet Digital PCR System, refer to the QX ONE Droplet Digital PCR System Instruction Manual (10000116512) and the QX ONE Software Instruction Manual for Standard Edition (10000116655) or Regulatory Edition (10000116656)

Visit **bio-rad.com/ddPCRsmxProbes** for more information.

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